Methods in Cell Biology

Edited by

DAVID M. PRESCOTT

VOLUME XV

Methods in Cell Biology

Edited by

DAVID M. PRESCOTT

DEPARTMENT OF MOLECULAR, CELLULAR AND
DEVELOPMENTAL BIOLOGY
UNIVERSITY OF COLORADO
BOULDER, COLORADO

VOLUME XV

ACADEMIC PRESS • New York San Francisco London
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 64–14220

ISBN 0–12–564115–X

PRINTED IN THE UNITED STATES OF AMERICA

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

BARBRO ANDERSSON, Department of Pathology, School of Medicine, Leahi Hospital, Honolulu, Hawaii (435)

STRATIS AVRAMEAS, Immunocytochemistry Unit, Pasteur Institute, Paris, France (387)

KARYN E. BIRD, Webb-Waring Lung Institute, University of Colorado Medical Center, Denver, Colorado (1)

L. M. BLACK, Department of Genetics and Development, University of Illinois, Urbana, Illinois (407)

MICHEL BORNENS, Department of Molecular Biology, Pasteur Institute, Paris, France (163)

JOSEPH E. CUMMINS, Department of Plant Sciences, The University of Western Ontario, London, Ontario, Canada (445)

RICHARD L. DAVIDSON, Division of Human Genetics, Children's Hospital Medical Center, and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts (325)

ALAN W. DAY, Department of Plant Sciences, The University of Western Ontario, London, Ontario, Canada (445)

T. EGE, Institute for Medical Cell Research and Genetics, Medical Nobel Institute, Karolinska Institute, Stockholm, Sweden (339)

A. J. FABER, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland (127)

S. FAKAN, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland (127)

PARK S. GERALD, Division of Human Genetics, Children's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts (325)

PETER J. GOLDBLATT, Department of Pathology, University of Connecticut Health Center, Farmington, Connecticut (371)

NICHOLAS K. GONATAS, Department of Pathology (Division of Neuropathology), University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania (387)

H. HAMBERG, Department of Pathology, Sabbatsberg Hospital, Stockholm, Sweden (339)

R. HANCOCK, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland (127)

REINHOLD G. HERRMANN, Institut für Botanik der Universität Düsseldorf, Düsseldorf, West Germany (177)

RICHARD H. HILDERMAN, Department of Biochemistry, Clemson University, Clemson, South Carolina (371)

MASAKATSU HORIKAWA, Division of Radiation Biology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan (97)

I. R. JOHNSTON, Biochemistry Department, University College, London, England (277)

JOSEPH R. KATES, Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York (359)

R. R. KAY, Imperial Cancer Research Fund, London, England (277)

CLAUDIA KENT, Department of Biochemistry, Purdue University, West Lafayette, Indiana (289)

ALOIS KNÜSEL, Department of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Zürich, Switzerland (89)

LEROY KUEHL, Department of Biochemistry, University of Utah, Salt Lake City, Utah (79)

CLIVE C. KUENZLE, Department of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Zürich, Zwitzerland (89)

M. LANNEAU, I.N.R.A. – Laboratoire de Physiologie de la Reproduction, Nouzilly, France (55)

TERESA H. LIAO, Department of Chemistry, Nutritional Science Program, University of Maryland, College Park, Maryland (381)

M. LOIR, I.N.R.A. – Laboratoire de Physiologie de la Reproduction, Nouzilly, France (55)

JOSEPH J. LUCAS, Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York (359)

F. MACHICAO, Max-Planck-Institut für Biochemie, Martinsried, West Germany (149)

GERARDO MARTÍNEZ-LÓPEZ, Virus Laboratory, Instituto Colombiano Agropecuario, Bogota, Colombia (407)

MARVIN L. MEISTRICH, Section of Experimental Radiotherapy, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas (15)

VOLKER MELL, Botanisches Institut der Universität Marburg, Marburg, West Germany (201)

MASAMI MURAMATSU, Department of Biochemistry, Tokushima University School of Medicine, Tokushima, Japan (221)

TOSHIO ONISHI, Department of Biochemistry, Tokushima University School of Medicine, Tokushima, Japan (221)

RAM PARSHAD, Department of Pathology, Howard University College of Medicine, Washington, D.C. (421)

N. R. RINGERTZ, Institute for Medical Cell Research and Genetics, Medical Nobel Institute, Karolinska Institute, Stockholm, Sweden (339)

WILLIAM S. RUNYAN, Department of Food and Nutrition, Iowa State University, Ames, Iowa (381)

MARLENE SABBATH, Department of Pathology, Lenox Hill Hospital, New York, New York (435)

TAKASHI SAKAMOTO,[1] Division of Radiation Biology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan (97)

R. J. SANDERSON, Webb-Waring Lung Institute, University of Colorado Medical Center, Denver, Colorado (1)

STEVEN D. SCHIMMEL, Department of Biochemistry, University of South Florida, College of Medicine, Tampa, Florida (289)

RUPERT SCHMIDT-ULLRICH, Tufts-New England Medical Center, Department of Therapeutic Radiology, Division of Radiobiology, Boston, Massachusetts (235)

JÜRGEN M. SCHMITT, Institut für Botanik der Universität Düsseldorf, Düsseldorf, West Germany (177)

DANIEL SCHÜMPERLI, Department of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Zürich, Switzerland (89)

HORST SENGER, Botanisches Institut der Universität Marburg, Marburg, West Germany (201)

E. SIDEBOTTOM, Sir William Dunn School of Pathology, Oxford University, Oxford, England (339)

[1] *Present address:* Research Laboratories, NISSUI Pharmaceutical Co., Ltd., 1805 Inarichō, Souka-shi, Saitama 340, Japan.

JOHANN SONNENBICHLER, Max-Planck-Institut für Biochemie, Martinsried, West Germany (149)

WILLIAM G. TAYLOR, Cell Physiology and Oncogenesis Section, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland (421)

J. E. THOMPSON, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada (303)

P. ROY VAGELOS, Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey (289)

DONALD F. H. WALLACH, Tufts-New England Medical Center, Department of Therapeutic Radiology, Division of Radiobiology, Boston, Massachusetts (235)

WAYNE WRAY, Department of Cell Biology, Baylor College of Medicine, Houston, Texas (111)

I. ZETL, Max-Planck-Institut für Biochemie, Martinsried, West Germany (149)

PREFACE

Volume XV of this series continues to present techniques and methods in cell research that have not been published or have been published in sources that are not readily available. Much of the information on experimental techniques in modern cell biology is scattered in a fragmentary fashion throughout the research literature. In addition, the general practice of condensing to the most abbreviated form materials and methods sections of journal articles has led to descriptions that are frequently inadequate guides to techniques. The aim of this volume is to bring together into one compilation complete and detailed treatment of a number of widely useful techniques which have not been published in full detail elsewhere in the literature.

In the absence of firsthand personal instruction, researchers are often reluctant to adopt new techniques. This hesitancy probably stems chiefly from the fact that descriptions in the literature do not contain sufficient detail concerning methodology; in addition, the information given may not be sufficient to estimate the difficulties or practicality of the technique or to judge whether the method can actually provide a suitable solution to the problem under consideration. The presentations in this volume are designed to overcome these drawbacks. They are comprehensive to the extent that they may serve not only as a practical introduction to experimental procedures but also to provide, to some extent, an evaluation of the limitations, potentialities, and current applications of the methods. Only those theoretical considerations needed for proper use of the method are included.

Finally, special emphasis has been placed on inclusion of much reference material in order to guide readers to early and current pertinent literature.

DAVID M. PRESCOTT

CONTENTS

Chapter 1

Cell Separations by Counterflow Centrifugation

R. J. SANDERSON AND KARYN E. BIRD

Webb-Waring Lung Institute,
University of Colorado Medical Center,
Denver, Colorado

I. Introduction

Counterflow centrifugation has been available as a technique for cell separation since 1948, when Lindahl first published an account of the method in *Nature* (Lindahl, 1948). In that same year, a patent was also filed in California by MacLeod (1948) for a counterflow centrifugation apparatus designed for a nonbiological application. More recently, Beckman Instruments developed and marketed a rotor which has been used by a number of investigators (Glick *et al.*, 1971; Flangas, 1974; Grabske *et al.*, 1974).

The original apparatus of Lindahl was exceedingly complex, and that of MacLeod was specialized for the concentration of particles of high specific gravity. The Beckman apparatus, however, is quite simple. We have modified